Computer Representation of the Synaptic Connectivity of Caenorhabditis elegans

Theodore B. Achacoso, M.D., Victor Fernandez, M.D.
Duc C. Nguyen, William S. Yamamoto, M.D.

Department of Computer Medicine
The George Washington University School of Medicine
Washington, D.C. 20037

Abstract

The paradigm for neural computing, which proceeds from theory, to abstraction, and on to design, may be reversed to pursue hardware development from designs based entirely on actual neural systems. The synaptic connectivity of the 302-neuron nematode, *Caenorhabditis elegans*, was modeled in a vertex adjacency matrix. The topological equivalents of this matrix, corresponding to certain neuroanatomic perspectives were analyzed both visually and using elementary matrix computations. While many methods have been devised to view and analyze the individual neuronal connections of parts of a nervous system, this method has provided a way to represent and analyze the synaptic connectivity pattern of an entire nervous system.

Introduction and Rationale

Neurocomputing has progressed logically within the framework of a true computing discipline, both in software and hardware development [1]. From a mathematical theory, it proceeds to construct a model on which experimentations and predictions can be made. After the latter process of abstraction, a neural network is designed, evaluated, and then applied in computers. This forward direction of neurocomputing, from theory to abstraction, and on to design has yielded impressive applications for pattern recognition, knowledge data bases, optimization computations, and robot control [2]. While this approach is extremely useful, it usually elects willfully to impose mathematical constraints on the behavior of a neuron, and thus, a neural network.

Reversal of the logical direction of neurocomputing to pursue hardware development involves the initial acceptance of a biological neural network design. Using the process of scientific abstraction, this design can be translated, wholly or in part, into an artificial or synthetic model on which experimentations can be made. Observation and analysis of the behavior of the synthetic translation may allow the discernment of the mathematical theory underlying the natural nervous system template. This approach from design, to abstraction, to deduction of theory, seeks to discover the rules governing biological neural networks. The initial acceptance of the design as the constant weakens the imposition of rules on neuronal behavior as the present evolutionary success of the natural design is already proof of effective innate neuronal control mechanisms. It is the discovery of the rules effecting such neuronal controls from the given design that now becomes a main activity of this direction.

The natural neural network design used for this investigation is the nervous system of *Caenorhabditis elegans*, a one millimeter, free-living soil nematode which exhibits the following general behavior: locomotion, feeding, mating, dauer formation in adverse environmental conditions, and response to mechanical, chemical, osmolar, and thermal stimuli. Three hundred eighty one (381) neurons comprise the entire nervous system of the male, while the hermaphrodite has three hundred two (302), the latter having been reconstructed in its entirety from serial electron micrographs and yielding detailed description of the structural connections of its nervous system [3].

Using the extensive neuroanatomic connection schematic diagrams, electron micrograph pictures, and the neuron list and labelling conventions provided in literature [3,4,5,6,7,8,9], translation of this natural nervous system to synthetic form started with a list of the three to five-letter codes for neurons of the hermaphrodite, with each neuron heading a sublist of all other neurons to which it was presynaptic, and of all other neurons from which it was postsynaptic, including counts of synaptic density.

Manipulation of the order of these neurons in a connection matrix corresponds to a topological deformation of the nervous system connectivity, and allows the prediction, observation, and analysis of the resultant synaptic connectivity pattern of the nervous system of *C. elegans* as a whole.

While detailed study of a nervous system usually focuses on the microscopic neuron to neuron anatomical connections, this method provides a representation of all the synaptic connections of an entire nervous system in one picture. As topological equivalents of the matrix are produced by manipulation of neuronal order, i.e, random, anatomic anteroposterior, anatomic left-right, order as to neuron type (as sensory, motor, or interneuron), and order respecting ganglionic location, different patterns of the same matrix are seen. Different views of one matrix containing all of the synaptic connections of an entire nervous system as elements allow various conclusions to be drawn.

Methodology

Comprehensive literature on hermaphroditic, wild-type *C. elegans* was surveyed and studied [3,4,5,6,7,8,9], allowing all neurons and their synapses to be listed in an electronic spreadsheet file. The number of synaptic connections of each neuron was manually counted from the schematic diagrams, serial electron micrograph pictures, and illustrations of the reconstructed worm in the published literature. In cases where only a neuron typical of a group was illustrated, segmental approximations were made to replicate the synaptic connections of the typical neuron at other locations in the neuraxis.

A computer program was written to manipulate the spreadsheet file, and this produced a text file which was the node-pair list of the directed graph of the *C. elegans* nervous system. From this node-pair list, computer programs were made to generate the vertex adjacency matrix of the directed graph. The vertex adjacency matrix for the directed graph G is an $m \times m$ matrix $A = (a_{ij})$ such that

$$a_{ij} = \begin{cases} n & \text{if } <v_i,v_j> \text{ is an arc of } G, \\ 0 & \text{otherwise,} \end{cases}$$

and where n equals synaptic weight or density [10]. Matrix elements (synapses) of the resultant matrix and of its topological equivalents were displayed as dots in an RGB monitor using EGA technology. The standard BASIC colors coded 1 to 15 were equated to synaptic weights or strengths, such that a blue dot (color code 1) indicated that the synapse occurred only once, and a high-intensity white dot (color code 15) indicated that the same synapse occurred 15 or more times. This convention was adapted because only a few neurons had synaptic densities of more than 15 in a single connection, although the highest density recorded in a single connection was 79. In instances where the interest was only in the synaptic connections and not in the synaptic densities, monochrome was used. Mathematically, the latter matrix elements are represented as

$$a_{ij} = \begin{cases} 1 \\ 0 \end{cases}$$

where all synaptic densities are given a value of one.

In these square matrix representations, each element of the matrix used the row index and the column index to identify the presynaptic and postsynaptic cell. If, for a given neuron, the row index was taken as equal to the column index, the principal diagonal of the matrix became equivalent to an anatomical axis of the animal. Topological equivalents of the vertex adjacency matrix were then produced by simple row-column exchanges that conformed to certain neuroanatomical perspectives, such as anteroposterior order, anatomic left-right order, order as to neuron type (as sensory, motor, and interneuron), and order as to ganglionic formation. The connection matrix and its topological equivalents were then analyzed visually and mathematically using elementary matrix computations, allowing the characterization of the nervous system as a whole.

Results and Discussion

Aside from the specific neuron to neuron connections, discussion of the 302-neuron, hermaphroditic *C. elegans* in literature included a description of some of its somatic and glial cell connections. Major anatomic omissions at present concern somatic connections to the pharyngeal, anal, and uterine musculature. Using the electronic spreadsheet, 2081 connections among the 302 neurons were listed, representing a total synaptic and somatic connection density of 6744 out of the 91,204 possible connections. Therefore, the directed graph represents a sparse matrix with only 2.28% occupancy. This connection density is less than those apparent in nervous systems of higher organisms, and appears to be within the range of easy analysis by computer-assisted design (CAD) methodology and may be feasible for physical analog fabrication using point to point wiring.

The 302 neurons are classified by morphology into 118 classes, 92 of which are catalogued by interclass connection [3]. The matrix representation of this network is also sparse, there being 461 connections out of a possible 8464 (5% occupancy), including the self-connection requisite for this type of representation. Figure 1 shows a graphical display of this matrix. By computing the transitive closure of the graph under Boolean multiplication, 5821 connections of 12 steps or less are found to strongly connect to all neuron classes, with the exception of some sensory neurons and some

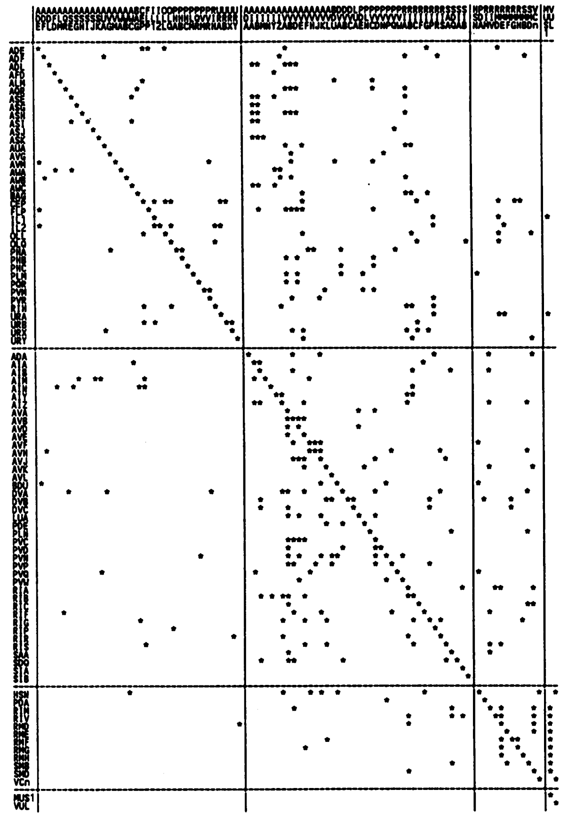

Figure 1. Matrix representation of the synaptic connections between neuron classes. Each asterisk (or matrix element) represents a synapse or synapses between a neuron class from the row (presynaptic class) and a neuron class from the column (postsynaptic class). Row and column labels correspond to neuron class names.

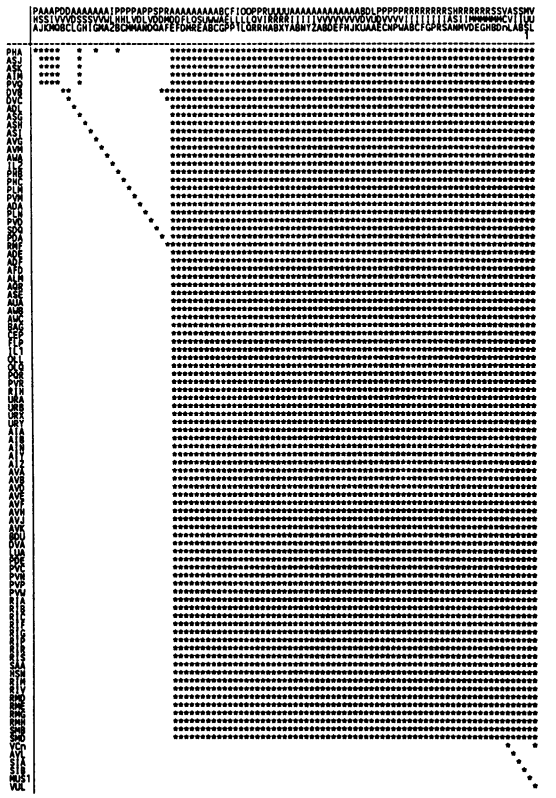

Figure 2. Transitive closure under Boolean multiplication of the matrix in Figure 1. To produce this matrix, further row-column exchanges were made to refine the resultant matrix after transitive closure. Through 12 synapses or less, every neuron might share in activity arising in any other neuron.

motor neurons (including end somatic cells). Further row-column exchanges show that nearly all neuron classes constitute the strongly connected subgraph (Figure 2). The nervous system of *C. elegans* is thus unitary as regards chemical synapses sorted by type, and demonstrates the possibility that through 12 synapses or less, every neuron shares in activity arising in any other neuron. In this analysis, no discrimination was made between excitatory and inhibitory connections.

Figure 3 shows a random arrangement of the neurons in the matrix. As expected, synapses are randomly distributed throughout the matrix, representing an unstructured nervous system connectivity pattern. Contrasted to this figure, various levels of synaptic organization can be recognized for each neuroanatomically based topological equivalent of the matrix.

The alphabetical labelling convention of the neurons provided in literature approximates the anteroposterior order of the neurons in the *C. elegans* nervous system. The matrix representation of this neuronal order is shown in Figure 4. Analysis of this figure is facilitated by equally subdividing the matrix into four submatrices, as left upper submatrix (LUS), left lower (LLS), right upper (RUS) and right lower (RLS). The main matrix diagonal passes through the LUS and RLS which contain the short tracts of the nervous system of *C. elegans*. The LUS contains the short anterior to anterior synaptic connections, such as those of the head, and the RLS contains the short posterior to posterior synaptic connections, such as those of the tail. It is visually observable that in areas where heightened neuronal activity is expected and where neuronal organization may involve ganglionic formations, such as in the head part of the worm (contained in the LUS), more synaptic connections are present, and connections are more

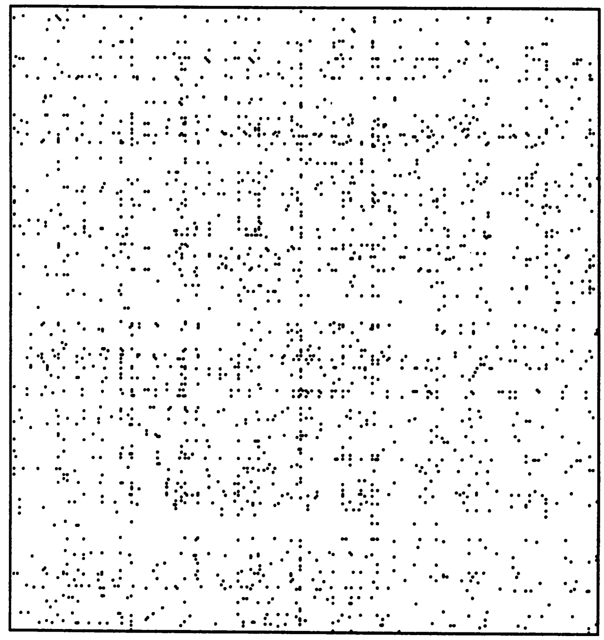

Figure 3. Random order. Each dot (or matrix element) represents a synapse or several synapses between a neuron from the row (presynaptic neuron) and a neuron from the column (postsynaptic neuron). Row and column labels (corresponding to neuron names) are in random order. Row and column labels are not shown in Figures 3 to 7.

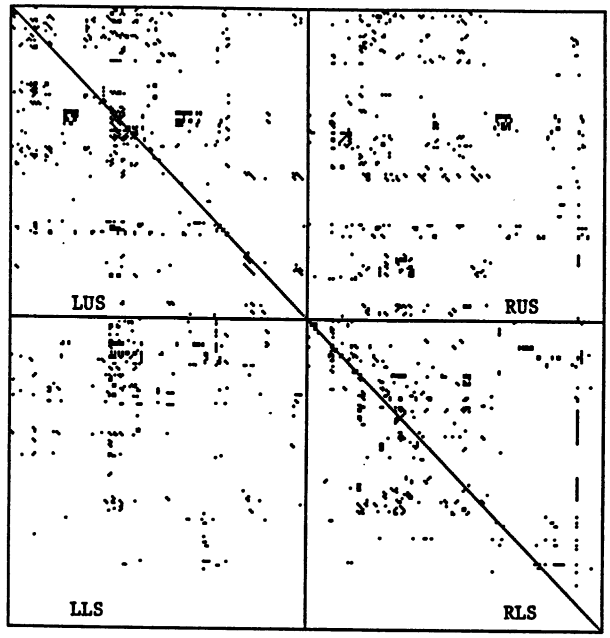

Figure 4. Anteroposterior order. Neurons represented by the row and column labels are arranged according to their anteroposterior appearance in the *C. elegans* neuropil. The right upper submatrix (RUS) contains the long anterior to posterior synaptic connections (descending tracts). See text for abbreviations, conventions for analysis, and discussion.

dense. There is distinct cephalization in the neuraxis. Elements in the RUS and the LLS represent the synaptic connections of the long tracts of the nervous system of *C. elegans*. The RUS contains the descending tracts (anterior to posterior connections) and the LLS contains the ascending tracts (posterior to anterior connections). If one were to engage in electromechanical fabrication of the *C. elegans* nervous system, short tracts (near the main diagonal) would indicate components to be grouped in proximity, and long tracts (in off-diagonals) would represent back plane or bus connections.

Arrangement of the neurons by anatomic laterality, left and right, in the matrix produces Figure 5. Homolateral synapses are contained in the LUS and RLS. Of special interest are the commissural connections contained in the RUS (left to right connections) and in the LLS (right to left connections). A simple connectivity count of the paired neurons shows that the left side of the nervous system receives less connections from the right (253), than vice-versa (268). Synaptic density count shows the same pattern, with the left side having less dense connections (732) than the right (801). Although nervous systems have been proven to be structurally and functionally asymmetrical and lateralized both in vertebrates [11,12,13,14] and in some invertebrates [15], analysis of this matrix demonstrates that neuroanatomical asymmetry, and perhaps functional lateralization, is primitive phylogenetically and resides deeply as the differences in neuronal connectivity and neuronal connection density of the left and right sides of the nervous system. The real challenge of this finding, however, is in its ethological significance.

When neurons are grouped as either sensory (S), motor (M), or interneurons (I) in the matrix, Figure 6 is produced. Nine submatrices are now identifiable, instead of four, and can be labelled according to the connections contained in each submatrix. As expected, the S to I (377 connections), I to I (447 connections), and I to M (368 connections) connections are dense. If the I to S (118 connections) and M to I (132 connections) are considered as sensory and motor feedback connections, respectively, the total connectivity count for the S to I and I to S set of connections becomes 495, and the total connectivity count for the I to M and M to I set of connections becomes 500. Nearly three-fourths of the entire 2081 connections of the *C. elegans* nervous system are then almost equally distributed in three layers, seemingly to validate current artificial neural network design used in neurocomputing. The other submatrices are sparse. The S to S submatrix which probably contains synapses for sensory modulation has 149 connections, the M to M which probably contains those for motor integration has 198, the S to M which probably has the monosynapses for direct reflexes has 164 connections, and the M to S submatrix which may be involved in proprioception has 25 connections.

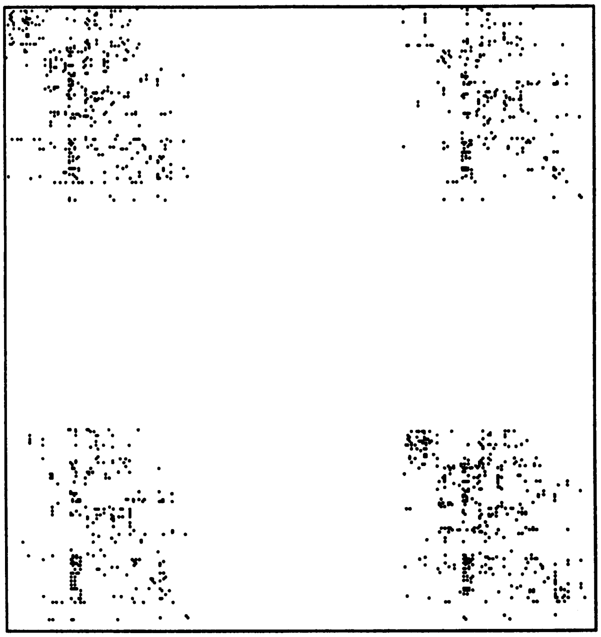

Figure 5. Order by anatomic laterality. Neurons represented by the row and column labels are ordered from left to right, as lateralized in the *C. elegans* neuropil. Homolateral synapses are located in the LUS and RLS while commissural connections are in the RUS (left to right connections) and LLS (right to left connections).

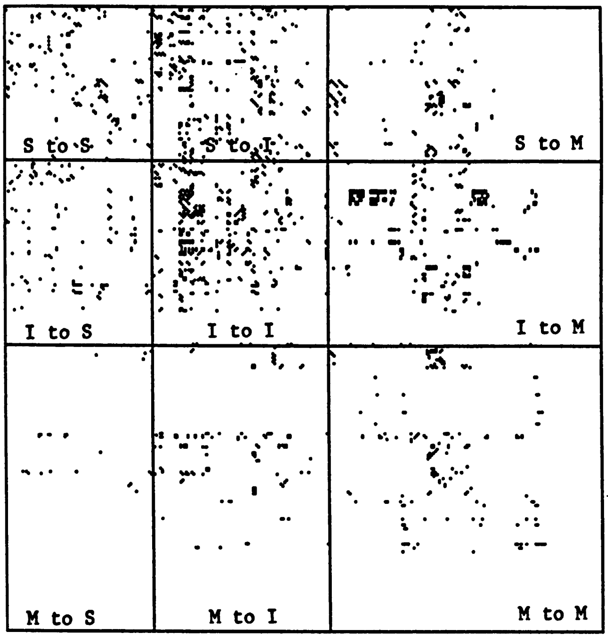

Figure 6. Order by neuron type: sensory, interneuron, or motor. Neurons represented by row and column labels are grouped as either sensory neurons (S), interneurons (I), or motor neurons (M). The neurons retain their anteroposterior order within each group. S to I, I to I, and I to M synaptic connections are dense.

C. elegans has nine defined ganglia. Categorizing neurons to their respective ganglia, and placing the ganglia in anteroposterior order in the connection matrix results in Figure 7. Actual quadrantic partitioning of the matrix, as was done for earlier analyses, seems to be unnecessary in this matrix since the matrix elements themselves have organized to form a discernible grid on the computer screen. The main diagonal now contains the ganglia (each one boxed off in the figure) with the intraganglionic connections, while the off-diagonals in the RUS and the LLS contain the interganglionic connections. This

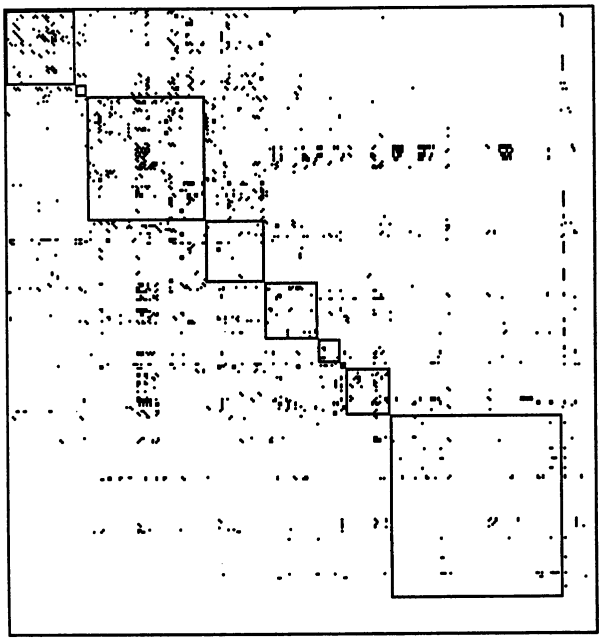

Figure 7. Ganglionic order. Neurons represented by row and column labels are grouped into their respective ganglia. The neurons are sorted alphabetically within each ganglion, and the ganglia are sequenced anteroposteriorly. Intraganglionic synaptic connections are contained in each box.

arrangement is significant in that it is a refinement of the neuroanatomic order presented in Figure 4. The significance of the reticulated appearance of the matrix is still being explored, and was initially suspected to be artifactual from the alphabetical arrangement of the neurons within each ganglion. Randomizing the order of the neurons within the ganglia, while maintaining ganglionic anteroposterior order in the matrix, however, still produces the same reticulation.

Summary and Conclusions

The paradigm for neural computing may be reversed to pursue hardware development from designs based entirely on actual neural systems. Simple computational tools, however, are necessary to abstract the anatomy into mathematical form.

The synaptic connectivity of the 302-neuron nematode, *Caenorhabditis elegans*, was modeled in a vertex adjacency matrix. The topological equivalents of this matrix, corresponding to certain neuroanatomic perspectives were analyzed both visually and using elementary matrix computations.

Random ordering of neurons in the connection matrix of the *C. elegans* corresponds to an unstructured nervous system. Anteroposterior order and ganglionic order of the neurons delineate the long and short tracts of the nervous system, and visually show that areas suspected of increased organization and function have more synaptic connections. These findings are useful for any future attempts to fabricate electromechanically the synaptic connectivity of this worm, as components with short tracts can be grouped together and long tracts can be grouped in a bus or back plane. The result of the left-right arrangement of neurons in the matrix suggests that lateralization exists, and may substantiate the dextro or sinistraversion of the worm during locomotion. Categorizing neurons as sensory (S), motor (M), and interneurons (I) in the matrix shows dense connections between S to I, I to I, and I to S, and this may provide the natural validation for the three-layer artificial neural network design.

While many methods have been devised to view and analyze the microscopic, individual neuronal connections of parts of a nervous system, the above method has provided a way to represent and analyze the synaptic connectivity pattern of an entire nervous system.

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